PLOUGMANN VINGTOFT & PARTNERS

27 APR. 2000

CRN/HM

PATENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

PLOUGMANN, VINGTOFT & PARTNERS A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

Date of mailing (day/month/year) 19 April 2000 (19.04.00)	
Applicant's or agent's file reference 21918 PC 1	**IMPORTANT NOTIFICATION**
International application No. PCT/DK00/00092	International filing date (day/month/year) 06 March 2000 (06.03.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 05 March 1999 (05.03.99)
Applicant HATTING-KS et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
05 Marc 1999 (05.03.99)	PA 1999 00317	DK	29 Marc 2000 (29.03.00)

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Marc Salzman
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/304 (July 1998)

003237489

PCT

06 MRS. 2000

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 21918 PC 1

Box No. I TITLE OF INVENTION

Determination of sperm concentration and viability, prediction of fertility in artificial insemination, and method for artificial insemination

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Hatting-KS
Oensvej 48
Hatting
DK-8700 Horsens

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

De Danske Kvægavlsforeninger
Udkærsvej 15
Skejby
DK-8200 Århus N

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Plougmann, Vingtoft & Partners A/S
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P.O. BOx 3007
DK-1021 Copenhagen K

Telephone No. +45 33 63 93 00

Facsimile No. +45 33 63 96 00

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III **FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CHRISTENSEN, Preben
Dalgas Boulevard 89, 1.th.
DK-2000 Frederiksberg

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

STENVANG, Jens Peter
Bransbjergvej 119
DK_2600 Glostrup

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AE** United Arab Emirates
- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria ...and utility model...
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus .....
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CR** Costa Rica .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic and utility model
- ☒ **DE** Germany ...and utility model...
- ☒ **DK** Denmark ...and utility model...
- ☒ **DM** Dominica
- ☒ **EE** Estonia ...and utility model...
- ☒ **ES** Spain .....
- ☒ **FI** Finland ...and utility model...
- ☒ **GB** United Kingdom
- ☒ **GD** Grenada
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **GM** Gambia
- ☒ **HR** Croatia .....
- ☒ **HU** Hungary .....
- ☒ **ID** Indonesia
- ☒ **IL** Israel .....
- ☒ **IN** India .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....and utility model...
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakhstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MA** Morocco .....
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia ...and utility model.....
- ☒ **SL** Sierra Leone .....
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **TZ** United Republic of Tanzania
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZA** South Africa .....
- ☒ **ZW** Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	[redacted]er of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 05.03.99 5 March 1999	PA 1999 00317	DK		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*
ISA / EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date (day/month/year)	Number	Country (or regional Office)
17.02.00	RS 104305	DK

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	4
description (excluding sequence listing part)	:	30
claims	:	6
abstract	:	1
drawings	:	7
sequence listing part of description	:	
Total number of sheets	:	47

This international application is **accompanied** by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: RS 104305 DK

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Copenhagen, 6 March 2000
Plougmann, Vingtoft & Partners A/S

Camilla Rendal Nielsen

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP ✓

US

29 SEP. 2000

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 21918 PC 1 ✓

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/DK00/00092 ✓	6 March 2000 (06.03.2000) ✓	5 March 1999 (05.03.99) ✓

Title of invention:
Determination of sperm concentration and viability for artificial insemination

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Hatting-KS
Oensvej 48
Hatting
DK-8700 Horsens

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

De Danske Kvægavlsforeninger
Udkærsvej 15
Skejby
DK-8200 Århus N

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CHRISTENSEN, Preben
Dalgas Boulevard 89, 1. th.
DK-2000 Frederiksberg

State *(that is, country)* of nationality: Denmark

State *(that is, country)* of residence: Denmark

[x] Further applicants are indicated on a continuation sheet.

International application No. DK00/00092

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

STENVANG, Jens Peter
Bransbjergvej 119
DK-2600 Glostrup

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
Denmark	Denmark

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/DK00/00092

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Plougmann, Vingtoft & Partners A/S
P.O. Box 3007
Sankt Annæ Plads 11
DK-1021 Copenhagen K

Telephone No.: +45 33 63 93 00

Facsimile No.: +45 33 63 96 00

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[X] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 4.

International application No.
[redacted]DK00/00092

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:		sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter	:	1	sheets	☐	☐
6. other *(specify)*	:		sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [x] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Copenhagen, 29 September 2000
Plougmann, Vingtoft & Partners A/S

Jan Simonsen (Assoc. No. 117)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PLOUGMANN VINGTOFT & PARTNERS

International Preliminary Examining Authority
European Patent Office
Erhardtstrasse 27
D-80298 München
Germany

BY TELEFAX
CONFIRMATION BY MAIL

PCT CHAPTER II

Copenhagen, 5 April 2001

International Patent Application No. PCT/DK00/00092
De Danske Kvægavlsforeninger
Analyse af sædceller
Our ref: 21918 PC 01

- 5 APR. 2001

Dear Sirs,

Referring to the written opinion dated 6 February 2001, we hereby submit a set of amended claims 1-44.

Amended claim 1 corresponds to previous claim 1 amended to include the features of previous claim 2 and further amended to include that the determination is an objective determination. Basis for this amendment is found in the description on page 11, lines 27-30, and further on page 26, lines 9-12 and on page 27, lines 19-23.

The Examiner has observed, cf. Section VIII 1. of the written opinion, that there seems to be an inconsistency between claim 1 and the last paragraph on page 6. In that respect Applicant has further amended claim 1 to read 'subjecting the semen sample or a diluted subsample of the semen sample to selective staining [of live and dead sperm cells] and determining the total concentration of the sperm cells and the proportion of live sperm cells by means of a detection means responsive to the selective staining'. There should hereby be no inconsistency between amended claim 1 and the description.

Amended claim 39 corresponds to previous claim 40 amended to include the features of previous claim 33 and are further amended to include that the insemination dose to be used for artificial insemination is selected on basis of the predicted likelihood of fertilizing.

Claim 40 corresponds to previous claim 41 and new claims 41-44 correspond to previous claims 34,35,37 and 38 amended to be dependent on amended claim 39.

COPENHAGEN OFFICE
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TELEFAX +45 33 63 96 00
e-mail pv@pv.dk
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JENS CHR. SKOUS VEJ 9
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DK - 8100 AARHUS C
TELEPHONE +45 87 39 18 00
TELEFAX +45 33 63 96 00
e-mail pv@pv.dk
www.pv.dk

Previous claim 2 has been deleted and the remaining claims have been renumbered accordingly.

The Examiner furthermore observes that it is not apparent what types of fluorochromes are defined by the terms SYBR 14 and MPR 71292, and whether these terms have a well recognised meaning. Product information for a LIVE/DEAD® Sperm Viability Kit (L-7011) is enclosed wherein the fluorochrome SYBR 14 is used as one of two dyes. The kit is manufactured by Molecular Probes, Inc. SYBR 14 is, thus, the commercial name of the specific fluorochrome used by the manufacturer of SYBR 14. Likewise, MPR 71292 is a fluorochrome manufactured by Molecular Probes, Inc., the properties of MPR 71292 differing from the properties of SYBR 14 in that the excitation of the MPR 71292 may be performed by means of a light source emitting light in a wavelength range about 543 nm where the excitation of SYBR 14 is performed by light source emitting light in a wavelength range about 488 nm, *cf.* the description on page 8, lines 4-12.

In D1, a process is disclosed wherein measurement of the fluorescent intensity F_x of different samples provides a measure of the cell concentration and the percentage of living cells in a sperm sample. The method comprises dissolving Propidium Iodide in a buffer and subsequently measuring the fluorescent intensity, adding the sperm sample and measuring the intensity, adding a membrane-permeabilizing agent and measuring the intensity, adding a buffer and a permeabilizing agent to the buffer and measuring the intensity, measuring the intensity of the pure buffer, adding the sperm mixture to the buffer and measuring the intensity, whereafter the cell concentration may be calculated. The process of D1 thus comprises measuring the emission intensity of *six* samples prepared in different ways so as to obtain a set of values from which the cell concentration and the percentage of living cells may be determined.

Nowhere in D1 is a method disclosed according to amended claim 1, wherein the determination of the total concentration of sperm cells and the proportion of live sperm cells are performed using the same sample or subsample and in the same determination routine.

D2 discloses a method of discriminating live and dead sperm by adding fluorescent colouring matters to stain all the sperm and the dead sperms, respectively. According to the fluorescence emitted from the head and the tail of a sperm, respectively, it is judged whether the sperm is a live sperm or a dead sperm. In D2 there is no disclosure as to the method of judging.

In D2, there is, thus, no disclosure of the invention according to amended claim 1 in the present application, wherein the objective determination of the total concentration of sperm cells and the proportion of live sperm cells are performed using the same sample or subsample and in the same determination routine. There is not in D2 any indication of how the total concentration could be determined in an objective way.

It is an advantage of the method according to the present invention that the sperm cell concentration and the proportion of live sperm cells are performed on the same sample or subsample, since multiple steps of addition, mixing and dilution during determination will add to the uncertainty of the procedure. Furthermore, since only a single sample or subsample need to be prepared, the method is readily adopted by the cattle artificial insemination industry, where the time and complexity of the method are crucial parameters, and where the laboratory facilities may be insufficient for too complex a method.

Furthermore, the method of the invention is an objective method which is substantially insensitive to operator dependent skills contrary to conventional microscopic methods which are heavily dependent on operator skills and experience. By the method of the invention, it is thereby possible to obtain uniform objective measurements for the total concentration of sperm cells and the proportion of live sperm cells.

According to the above-mentioned, Applicant finds that the subject-matter of amended claim 1 is novel and involves an inventive step so that the requirements of Art. 33(2) and Art. 33(3)PCT are met.

Applicant has amended claim 40 according to the remarks from the Examiner regarding novelty of claim 40, and Applicant finds that the subject-matter of amended claim 40 is novel and involves an inventive step so that the requirements of Art. 33(2) and Art. 33(3)PCT are met.

It is proposed that correction of defects in the form be postponed till the Examiner has indicated that the new claims have been found to be allowable.

In case the Examiner does not agree that the new claims are properly based on the documents originally filed, and that the invention defined in the new claims is novel and involves an inventive step, a telephone interview with the Examiner pursuant to Rule 66.6 PCT or a second Written Opinion is requested prior to the issuance of a preliminary examination report.

Please confirm receipt by enclosed form 1037.

Yours sincerely,

Plougmann, Vingtoft & Partners

CRN

Camilla Rendal Nielsen

Form 1037
Amended claims 1-44
Product information for LIVE/DEAD® Sperm Viability Kit (L-7011)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PLOUGMANN & VINGTOFT A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
25 January 2002 (25.01.02)	
Applicant's or agent's file reference 21918 PC 1	
International application No. PCT/DK00/00092	**International filing date** (day/month/year) 06 March 2000 (06.03.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

PLOUGMANN, VINGTOFT & PARTNERS A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
Denmark

State of Nationality | State of Residence

Telephone No. 45 33 63 93 00

Facsimile No. 45 33 63 96 00

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

PLOUGMANN & VINGTOFT A/S
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
Denmark

State of Nationality | State of Residence

Telephone No. 45 33 63 93 00

Facsimile No. 45 33 63 96 00

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Anne KARKACHI
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PA IT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 November 2000 (13.11.00)	
International application No. PCT/DK00/00092	**Applicant's or agent's file reference** 21918 PC 1
International filing date (day/month/year) 06 March 2000 (06.03.00)	**Priority date** (day/month/year) 05 March 1999 (05.03.99)
Applicant CHRISTENSEN, Preben et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 September 2000 (29.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

DK0000092

PATENT COOPERATION TREATY

& PARTNERS

08 FEB. 2001

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT CRW/LSC

To:

PLOUGMANN; VINGTOFT & PARTNERS A/S
Sankt Annae Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	06.02.2001
Applicant's or agent's file reference: 21918 PC 1	**REPLY DUE** **within 2 month(s)** from the above date of mailing

Internationcal application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00092	06/03/2000	05/03/1999

International Patent Classification (IPC) or both national classification and IPC

G01N15/14

Applicant

HATTING-KS et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 05/07/2001.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Komenda, P

Formalities officer (incl. extension of time limits)
Conner, M
Telephone No. +49 89 2399 2241

Form PCT/IPEA/408 (cover sheet) (January 1994)

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-30 as originally filed

Claims, No.:

1-41 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrialapplicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1,40,41
Inventive step (IS)	Claims	
Industrial applicability (IA)	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section V:

1. Reference is made to the following documents:

   D1 = GB-A-2 214 518
   D2 = PAJP, vol. 1997, no. 4, 30.04.1997 & JP 08 332098 A

2. Independent claim 1 as presently worded appears to be anticipated by D1 (Article 33(2) PCT) which reveals a method for determining the total concentration of sperm cells in a semen sample and the proportion of live sperm cells therein. The method of D1 is also based on the staining of live and dead cells using a fluorescent agent (see abstract).

3. Document D2 reveals a method of determining live and dead sperm cells using fluorescent agents which selectively stain said two types of sperm cells. The difference between the method of D1 and that according to claim 1 is the fact that the former does not mention determination of total concentration of sperm cells. It appears however, that once said two types of cells are discriminated, the skilled person would be able to apply well known counting methods in order to additionally determine the concentration of said cells. Such a modification of the method of D2 can thus be applied by the skilled person in accordance with circumstances without any need of performing an inventive activity (Article 33(3) PCT).

4. The method of independent claim 40 is not novel. Artificial insemination methods are well known in the art. In all said methods, insemination doses which have a likelihood of fertilising a female animal are used. The fact that the insemination dose has been analysed by the method of claims 33-35 does not impose any limitation to said conventional AI method since analysis apparently is performed before AI so that the insemination doses used are not distinguishable one from the other. Moreover, claim 40 does not mention any specific threshold value.

   Similar considerations apply to claim 41.

5. Should the applicant regard some particular matter of a dependent claims as patentable, an independent claim should be filed taking account of Rule 29(1) EPC. The applicant should also indicate in the letter of reply the difference of the subject-matter of the new claim vis-à-vis the state of the art and the significance thereof.

Section VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

2. The independent claims are not in the two-part form in accordance with Rule 6.3(b) PCT, which in the present case would be appropriate, with those features known in combination from the prior art (document D1) being placed in a preamble (Rule 6.3(b)(i) PCT) and with the remaining features being included in a characterising part (Rule 6.3(b)(ii) PCT).

3. In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT).

   If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Section VIII:

1. There appears to be an incon sistency between the description and claim 1. According to claim 1 live and dead sperm cells are selectively stained. According to page 6, final paragraph of the description however, selective staining is

obtained by using a dye which stains all cells combined with a dye which stains only dead cells. Amendment in this respect is thus required.

2. It is at present not apparent what type of fluorochromes are defined by SYBR-14 and MPR71292, respectively, and whether these terms have a well recognised meaning in the particular art.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PLOUGMANN; VINGTOFT & PARTNERS A/S
Sankt Annae Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	31.05.2001
Applicant's or agent's file reference 21918 PC 1	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00092	06/03/2000	05/03/1999

Applicant
HATTING-KS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Schuster-Kaechele, W Tel.+49 89 2399-2281

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21918 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00092	06/03/2000	05/03/1999
International Patent Classification (IPC) or national classification and IPC G01N15/14		
Applicant HATTING-KS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/2000	31.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Komenda, P Telephone No. +49 89 2399 2777

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK00/00092

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-30 as originally filed

Claims, No.:

1-44 with telefax of 05/04/2001

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-44
	No:	Claims	
Inventive step (IS)	Yes:	Claims	32-44
	No:	Claims	1-31
Industrial applicability (IA)	Yes:	Claims	1-44
	No:	Claims	

2. Citations and explanations
   see separate sheet

Section V:

Reference is made to the following document:

D1 = PAJP, vol. 1997, no. 4, 30.04.1997 & JP 08 332098 A

N: Document D1 reveals a method of determining live and dead sperm cells using fluorescent agents which selectively stain said two types of sperm cells. The difference between the method of D1 and that according to claim 1 is the fact that the former does not mention determination of total concentration of sperm cells (Article 33(2) PCT).

It should be mentioned here, that since D1 relies on the emitted fluorescence of the selectively stained cells, it appears to be implicit that also "detection means responsive to the selective staining" are used.

IS: With respect to the above distinguishing feature of claim 1, it appears, that once said two types of cells are discriminated, the skilled person would be able to apply well known counting methods in order to additionally determine the concentration of said cells. Such a modification of the method of D1 can thus be applied by the skilled person in accordance with circumstances without any need of performing an inventive activity (see in this respect also page 7, top) (Article 33(3) PCT).

At present it is not apparent, for which technical problem the features of claims 2 to 31 would provide an inventive solution as required by Article 33(3) PCT.

The use of the method of claim 1 for artificial insemination as defined in claims 32-44 is neither disclosed nor indicated in D1.

IA: Industrial applicability is acknowledged (Article 33(4) PCT).

WY

ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
21918 PC 1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/DK 00/ 00092	06/03/2000	05/03/1999
Applicant		
HATTING-KS et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

   DETERMINATION OF SPERM CONCENTRATION AND VIABILITY FOR ARTIFICIAL INSEMINATION

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 00/00092

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N15/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 214 518 A (INNOFINANCE ALTALANOS INNOVACIOS PENZINTEZET) 6 September 1989 (1989-09-06) figure 1 page 7, line 5 -page 10, line 21 page 5, line 9 -page 6, line 4 page 3, line 10 - line 25 page 2, line 12 - line 15 page 1, line 4 - line 14 --- -/--	1,7-10, 33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2000	23/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thomas, R.M.

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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International Application No
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CLAIMS

1. A method for the determination of the total concentration of sperm cells in a semen sample and the proportion of live sperm cells therein, comprising subjecting the semen sample or a diluted subsample of the semen sample to selective staining of live and dead sperm cells and determining the total concentration of the sperm cells and the proportion of live sperm cells by means of a detection means responsive to the selective staining.

2. A method according to claim 1, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed using the same sample or subsample and in the same determination routine.

3. A method according to claim 2, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed substantially simultaneously.

4. A method according to claim 3, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed in the same determination operation.

5. A method according to any of claims 1-4, wherein the selective staining comprises a staining which stains all sperm cells combined with a staining which selectively stains dead cells.

6. A method according to claim any of the preceding claims, wherein any dilution of the sample has been performed using a diluent which sustains viability of the sperm cells during the determination.

7. A method according to any of the preceding claims, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells and dead sperm cells, the fluorescent quality or qualities of live cells being distinguishable, by the detection means, from the fluorescent quality or qualities of dead sperm cells, and the determination is performed by selective counting of cells of each fluorescent quality.

8. A method according to any of the preceding claims, wherein the proportion of dying sperm cells is also determined, the selective staining being adapted to allow distinction, by the detection means, between dying sperm cells and on the one hand dead sperm cells and on the other hand live sperm cells.

9. A method according to claim 8, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells, dead sperm cells and dying sperm cells, the fluorescent quality or qualities of live sperm cells, dead sperm cells and dying sperm cells being distinguishable from each other by the detection means, and the determination is performed by selective counting of cells of each fluorescent quality.

10. A method according to any of claims 7-9, wherein the fluorochromes are fluorochromes binding to DNA.

11. A method according to claim 10, wherein the fluorochromes comprise a fluorochrome capable of selectively staining dead or dying sperm cells, this fluorochrome being capable of entering a sperm cell through a leaking or defect plasma membrane, but substantially incapable of entering a sperm cell having an intact plasma membrane, and another fluorochrome capable of staining all sperm cells, this fluorochrome being capable of entering a cell through an intact cell membrane.

12. A method according to any of claims 7-11, wherein the excitation of the fluorochromes is performed by means of light in the wavelength range about 488 nm, the fluorochrome staining all sperm cells being SYBR-14, and the fluorochrome staining the dead or dying sperm cells being propidium iodide.

13. A method according to any of claims 7-11, wherein the excitation of the fluorochromes is performed by means of light in the wavelength range about 543 nm, the fluorochrome staining all sperm cells being MPR71292, and the fluorochrome staining the dead or dying cells being ethidium-homodimer-2, EHD2.

14. A method according to any of claims 7-13, wherein the fluorochrome staining all sperm cells is used in total concentrations below standard total concentrations conventionally applied for such fluorochromes.

15. A method according to any of claims 7-14, wherein the fluorochrome staining all sperm cells is used in total concentrations in the range from 25 to 75 nanomolar.

16. A method according to claim 15, wherein the fluorochrome staining all sperm cells is used in total concentrations about 50 nanomolar.

17. A method according to any of claims 7-16, wherein the staining of the sperm cells is performed at a temperature below 35°C.

18. A method according to claim 17, wherein the staining of the sperm cells is performed at a temperature of at the most 30°C.

19. A method according to claim 18, wherein the staining of the sperm cells is performed at a temperature between 15°C and 25°C.

20. A method according to claim 19, wherein the staining of the cells is performed at room temperature.

21. A method according to any of the preceding claims, wherein the sample or subsample is combined with an internal concentration standard means, and the determination of the total concentration of the sperm cells and the proportion of live sperm cells are performed simultaneously by means of a detection means responsive to the selective staining and to the internal concentration standard means.

22. A method according to claim 21, wherein the internal concentration standard means is constituted by standardisation particles, the standardisation particles being added in a predetermined number per weight or volume amount of the sample or subsample.

23. A method according to claim 21 or 22, wherein the standardisation particles are fluorescent particles having a fluorescent quality distinguishable from the fluorescent qualities of the live sperm cells, dead sperm cells, and dying sperm cells.

24. A method according to any of claims 21-23, wherein the detection means comprises a flow cytometer.

25. A method according to any of claims 21-23, wherein the detection means comprises a laser scanning cytometer.

26. A method according to any of claims 21-25, wherein the size and total sperm cell concentration of a subsample are adapted so that the number of sperm cells corresponds to between one tenth and ten times the number of standardisation particles.

27. A method according to claim 26, wherein the size and total sperm cell concentration of the subsample are adapted so that the number of sperm cells corresponds to between one quarter and four times the number of standardisation particles.

28. A method according to claim 27, wherein the size and total sperm cell concentration of the subsample are adapted so that the number of sperm cells corresponds to between half and twice the number of standardisation particles.

29. A method according to any of claims 21-28, wherein the diluent is a diluent containing protein.

30. A method according to claim 29, wherein the protein is BSA.

31. A method according to any of claims 21-28, wherein the diluent is a diluent containing polyvinyl alcohol.

32. A method according to any of the preceding claims, wherein the determination of the total concentration of the sperm cells and the proportion of live sperm cells are determined as a mean value of the determination of the total concentration of the sperm cells and the proportion of live sperm cells performed on two or more subsamples of a semen sample.

33. A method for predicting the likelihood of fertilizing a female animal by artificial insemination with an insemination dose, comprising determining the total concentration of sperm cells in the semen sample from which the insemination dose is taken or is to be taken, and the proportion of live sperm cells therein by a method according to any of claims 1-32, and including the thus determined total concentration of the sperm cells in

the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample, in the parameters on the basis of which the likelilhood of fertilizing the animal is predicted.

34. A method according to claim 33, wherein the likelihood of fertilizing the female animal is predicted on the basis of the determined total concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample.

35. A method according to claim 33 or 34, wherein the prediction of the likelihood of fertilizing the female animal is performed on the basis of statistically significant correlations between fertility data obtained in insemination experiments with several female animals and data indicating the total concentration of the sperm cells in the semen sample used in the insemination experiments and the proportion of live sperm cells therein, and/or data indicating the concentration of live sperm cells therein.

36. A method according to any of claims 33-35, wherein the female animal is a multiparous animal, and the number of offspring resulting from the fertilization is also predicted.

37. A method according to any of claims 33-36, wherein the semen sample is a fresh ejaculate.

38. A method according to any of claims 33-36, wherein the semen sample is a frozen insemination dose, the sample being thawed before being subjected to the determination method.

39. A method according to claim 38, wherein data obtained by the determination method performed on the fresh ejaculate from which the insemination dose was taken are included together with data obtained by the determination method performed on the insemination dose.

40. A method for artificial insemination of a female animal, comprising using, for the insemination, an insemination dose having a predicted likelihood of fertilizing the animal,

as predicted by the method according to any of the claims 33-35 and 37-39, above a predetermined discrimination likelihood.

41. A method according to claim 40, wherein the female animal is a multiparous animal, and the insemination dose is an insemination dose having a predicted likelihood of resulting in a number of offspring above a predetermined discrimination number.